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Studies on the Extraction of Gelatin From Shark Skin and Skate Skin

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Chinese Abstract[1]

Gelatin is a derived protein that is produced by the action of an acid or alkali on collagen, the major constituent of the skin, muscles, sinews and bones of which animals are made. Taiwan has a comparatively high output of sharks and skates, which have a thick skin that contains abundant [raw material for the production of][2] gelatin[3]. The present study investigates the principle of treating these fishes' skin by an alkaline method that enables the collagen to be transformed into gelatin as a result of denaturation that occurs on the application of an alkali. With this method, a simple, convenient heating procedure is employed

[1] This is a translation of the original Chinese abstract. A somewhat different English-language abstract accompanied the paper and has been appended. (Translator's note.)

[2] Square brackets indicate inferences or clarifications that have been added by the translator. (Translator's note.)

[3] Literally, the Chinese text states that the "skin layers" or "cortex" of these fishes "is thick and rich in gelatin." Since, of course, gelatin *per se* does not exist in nature, it is being assumed that the intended meaning, here and in a similar passage below, was that ample amounts of gelatin can be obtained by processing the materials in the outer layers of their skin. (Translator's note.)

under optimal conditions to extract the gelatin and thereby increase the added value of the fishery products.

1. Extraction of Gelatin From These Fishes' Skins

   A. The optimal conditions for the extraction of shark-skin gelatin are to soak the shark skin in saturated calcium hydroxide solution[4] for three weeks and then carry out the extraction for 30 minutes at pH 6.8 and a temperature of 80° C.

   B. The optimal conditions for the extraction of skate-skin gelatin are to soak the skate skin in saturated calcium hydroxide solution for three weeks and then carry out the extraction for 30 minutes at pH 6.8 and a temperature of 70° to 80° C.

2. Bleaching of Gelatin [Derived From] These Fishes' Skins

   Shark-skin gelatin or skate-skin gelatin is bleached with 2% hydrogen peroxide, then dried in air heated to 80° C. This yields snow-white gelatin powder.

3. Enzyme Treatment of Gelatin [Derived From] These Fishes' Skins

   A. A 20% solution of shark-skin gelatin is treated for one hour with 0.01% papain at pH 5.0 and a temperature of 35.5° C. After the enzymatic activity has been destroyed [by subjecting the enzyme-treated solution] to a temperature of 100° C for 10 minutes, the [solution] is dried. This yields a food binder of excellent quality.

   B. A 20% solution of shark-skin gelatin is treated for one hour with 0.01% pancreatin at pH 7.5 and a temperature of 35.5° C. After the enzymatic activity has been destroyed [by subjecting the enzyme-treated solution] to a temperature of 100° C for 10 minutes, the [solution] is dried. This, too, yields a food binder of excellent quality.

4. The Quality of the Gelatin [Derived From] These Fishes' Skins

   A comparative analysis of their respective amino acid compositions revealed that gelatin made from shark skin and gelatin made from skate skin are both of higher quality than gelatin that is produced from pork skin.

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[4] Or "saturated lime water." (Translator's note.)

Introduction

Gelatin (*1, 2*) is a derived protein that is produced by the action of an acid or alkali on collagen, the major constituent of the skin, muscles, sinews and bones of which animals are made. As a rule, gelatin is a granular or powdery substance that is light yellow to yellow in color and tastes something like meat juice. The general composition (*1*) of gelatin is as follows: In 100 g of gelatin, there is 85.5 g of protein, 0.5 g of fat, 1.5 g of ash, 0 g of carbohydrates, 380 mg of calcium (Ca), 0 mg of sodium (Na), 6 mg of phosphorus (P), 1.8 mg of iron (Fe), and 388 cal of [food] energy. As was stated above, gelatin is known to be an excellent food product that has a very high protein content, and it also is a colloidal compound that exhibits superior properties as regards its isoelectric point for the formation of gels (*1, 2*). [These qualities] have led to its being developed as a food product that is suitable for use in outer space (*1*), and it has also attracted the attention of ordinary people. Gelatin (*1*) has always been used in the manufacture of jelly, marshmallows, nougat, yogurt, ham, processed milk, cans[5], etc., and it has also been utilized in other capacities, for example, as a clarifying agent [in the production of] alcoholic beverages. Gelatin thus is intimately related to the manufacture and cooking of foods.

Another notable fact is that gelatin can be used in the manufacture of microcapsules (*1*), to encapsulate inorganic or organic gases, liquids, solids, dyestuffs, pigments, detergents, catalytic agents, rust inhibitors, fuels, solvents, light-sensitive

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[5] Sic. Should be "canned foods," or something similar? (Translator's note.)

materials, binding agents, agricultural chemicals, fertilizers, fodder, fire-extinguishing agents, enzymes, medicines and chemical reagents, cosmetics, perfumes, foods, food additives, and so on. Its applications, in other words, are legion.

Recognizing the very high quality of pork-skin gelatin and its relatively wide range of applications, the researcher who conducted the experiments that are being reported here realized that the fishery products shark skin and skate skin would have enhanced added value if gelatin could be extracted from them, and that [such an accomplishment] could not only extend the application areas available to the fishery industry but would also result in a higher level of profit for it. Moreover, at the present time — a time when attempts are being made to utilize fishery products in a variety of areas — sharks and skates are being produced by Taiwan in rather sizable numbers. According to information presented in the annual reports for fisheries in the Taiwan area that were put out by the Fisheries Bureau of the Province of Taiwan for the years 1976 and 1977 (*3, 4*), the catch of sharks [during those years] came to 53,892 metric tons while that of skates was 6,113 metric tons. These figures made /p. 38 it evident that it would be worthwhile to boost the amount of research that was being done on the exploitation and utilization [of this resource]. At present only skates of a large size are consumed by some people as food or subjected to processing; the smaller ones are treated as common, lowly types of fish and sell for a cheap price, i.e., for no more than a few [New Taiwan] Dollars per kilogram. However, shark skin and skate

skin are thick and [potentially a] rich [source of] gelatin[6]. The collagen [in the skin layers] can be transformed into gelatin by denaturation achieved through the use of an acid or alkaline process. If, through the implementation of this principle, gelatin can then be extracted by applying a simple, convenient heating method under optimal conditions, the economic value [of these fishes] will certainly be magnified. Moreover, another effective way to exploit the gelatin that can be obtained from shark skin is to treat it with proteolytic enzymes for the purpose of manufacturing binders of improved quality that can be widely utilized in ordinary food products or fishery food products (for example, refined products).

Experimental Materials and Methods (*1, 2*)

Commercially purchased shark skins and the freshly peeled skins of skates just offloaded from a ship were used as the experimental materials. Other materials that were employed included such chemical reagents as lime and hydrochloric acid. The hydrochloric acid was a special-grade reagent made by Wako Pure Chemicals, Ltd., of Japan, while the lime was the ordinary commercial product. The papain (Art. 7147, Lot No. 4156064, 1 Anson E/g, 12000 E/g) and pancreatin[7] (the enzyme known under this commercial name is a freeze-dried product of pancreatic juice, Art. 7130, Lot No.

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[6] See footnote No. 3. (Translator's note.)

[7] Although the Chinese text shows "pancreatin" in English, it actually uses the character combination for trypsin, not the one that is usually seen for pancreatin. This anomaly occurs throughout the text. (Translator's note.)

1958435, a mixture composed of 1000 E/g protease, 4000 E/g lipase and 8000 E/g amylase) were both made by the [E.] Merck Co., and the standard amino acid that was utilized for the amino acid analyses was likewise a product of [E.] Merck Co. The hydrogen peroxide (H_2O_2) that was used in bleaching the gelatin was a special-grade reagent from [E.] Merck Co. that contained 30% H_2O_2.

The shark skin or skate skin must be treated before the gelatin can be extracted. Two types of method — an alkaline method or an acid method — can be used to treat them (*1, 2*). The alkaline approach was adopted in these studies.

A. Pretreatment of the Shark Skin and Skate Skin

Shark skins and skate skins that had been thoroughly washed until they were clean were separately soaked in saturated calcium hydroxide solution at room temperature (approximately 25° C) for a period ranging from one to seven weeks (in the case of shark skin) or one to five weeks (skate skin). Each week that the skins were being soaked, some of them were removed [from the saturated calcium hydroxide solution] and rinsed for a day with running tap water, then re-soaked for six to eight hours in 1 N hydrochloric acid to neutralize the alkali. After the skins had been removed [from the hydrochloric acid], they were again rinsed for a day with running tap water to cause them to swell up. The resulting fish skins constituted the "pretreated skins." All of the above operations were carried out at room temperature.

B. Extraction of Gelatin From the Shark Skin and Skate Skin and Determination of Its Gel Strength (5, 6)

The pre-treated shark skins were homogenized in a homogenizer before gelatin was extracted from them at a temperature of either 50°, 60°, 70°, 80°, 90°, 100°, 110°, 120° C, etc. The amount of shark skin that had to be utilized at each extraction temperature was 50 g; the extraction time was 30 minutes and the pH 6.8. In addition, the pre-treated skate skins were homogenized in a homogenizer, then gelatin was extracted from them at a temperature of 50°, 60°, 70°, 80°, 90°, etc. The amount of skate skin that had to be utilized at each extraction temperature was also 50 g, and the extraction time was 30 minutes and the pH 6.8. Distilled water was added to each of the gelatin solutions that were obtained by extracting the shark skins or skate skins at the various temperatures cited above, until a final volume of 100 mL was reached. The gel strengths [of these gelatin solutions] were then determined at 18° C utilizing Okada's instrument for measuring gel strength (*5*).

C. The Ratio of Recovery of Gelatin From the Shark Skin or Skate Skin

Sharks skins that had been pretreated by soaking for one to seven weeks, or skate skins that had been pretreated by soaking for one to five weeks, were homogenized using a homogenizer. Then gelatin was extracted from 50 g portions of these skins, at various temperatures ranging from 50° C to 120° C in the case of the shark skin or 50° C to 90° C in the case of the skate skin. The gelatin thus extracted from the shark skin, as well as the shark skin left behind as a residue following the

extraction process, were dried until they reached a constant weight, and so were the gelatin that had been extracted from the skate skin and the skate skin that remained after the extraction. This was done in order to find the ratios of recovery of gelatin from the shark skin or skate skin.

D. Extraction of Gelatin From Pork Skin and Determination of Its Gel Strength (4, 5)

Commercially obtained pork skins and shark skins were pretreated by the same method, then soaked for one to four weeks at room temperature in saturated calcium hydroxide solution. The pork skins and shark skins that were removed for testing each week were processed by identical methods, then homogenized in a homogenizer before being extracted for 30 minutes at pH 6.8 at a temperature of either 50°, 60°, 70°, 80°, 90°, 100°, 110°, or 120° C, etc. The amount of homogenized pork skin that was needed for the extraction at each of the stipulated temperatures was 50 g. Distilled water was added to the gelatin solutions that were obtained by extracting the pork skins to make up a final volume of 100 mL, then the gel strengths of the gelatin [solutions] derived from pork skin were determined by the same method described above. The resulting gel strength values were used for comparative purposes.

E. Enzymatically Digested Shark-Skin Gelatin

(1) Shark-Skin Gelatin Digested With Papain (*7, 8*)

A 20% solution of gelatin obtained from shark skin was digested with 0.01% papain, under reaction conditions whereby the pH was 5.0 and the temperature was

35.5° C. The gel strength measurements were made after 0, 1, 2, 3, and 4 hours, with one reading being taken each hour. A temperature of 20° C was employed for the gel strength determinations.

(2) Shark-Skin Gelatin Digested With Pancreatin (*7, 8*) /p. 39

A 20% solution of gelatin obtained from shark skin was digested with 0.01% pancreatin[8], under reaction conditions whereby the pH was 7.5 and the temperature was 35.5° C. The gel strength measurements were made after 0, 1, 2, 3, and 4 hours, with one reading being taken each hour. A temperature of 20° C was employed for the gel strength determinations.

F. Amino Acid Analysis of Gelatins Derived From Shark Skin and Skate Skin (9, 10)

Gelatin obtained from shark skin and from skate skin was analyzed using the Beckman 120B Amino Acid Analyzer. The analytical steps were as follows.

(1) Weighing and Dispensing of the Analysis Sample: A 5–10 mg portion of shark-skin gelatin or skate-skin gelatin was weighed and placed in a 15 mL amino acid analysis test tube made of hard glass. To this test tube was then added 3 mL of 6 N hydrochloric acid. The area of the test tube located one-third of the distance from its top was then roasted in a flame and drawn out to give the tube a narrow neck, in order to facilitate vacuum sealing of the test tube by means of a vacuum pump.

(2) Freezing of the Analysis Sample: A test tube that had an elongated neck and

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[8] Sic. See footnote 7. (Translator's note.)

contained the sample to be analyzed was placed in a beaker, into which had been packed a cold medium (such as acetone or methanol) and dry ice. The analysis sample was then left to freeze until it had solidified.

(3) Evacuation and Sealing of the Test Tube: The test tube containing the frozen sample to be analyzed was hooked up to a vacuum pump by means of rubber tubing. When drawing the air out of the test tube, air that had been dissolved in the analysis sample posed the main problem to be concerned about. Since the act of drawing out the air caused the analysis sample to be bumped in the course of evacuating the test tube, it was necessary to place the bottom of the test tube containing the sample in a beaker that had been packed with a cold medium and dry ice. This made the frozen analysis sample dissolve slowly, until the point was reached where no more air was dancing about [in the sample]. The attainment of a vacuum in the test tube was signified when a metallic sort of sound could be heard when a finger was rapped against the tube; this sound could be produced after the vacuum pump had been used to evacuate the test tube for 20 minutes. When this point was reached, the vacuum pump was kept in operation so as to continue to draw out air while a flame was simultaneously applied to the thin neck portion of the glass tube until it had been completely sealed shut.

(4) Hydrolysis: The sealed test tube containing the sample to be analyzed was placed in a copper tube and then inserted into a decomposing oven, where the sample was hydrolyzed at 110° C for 22 hours. Provided that all of the operations proceeded

smoothly to completion without a hitch, the values obtained in the amino acid analysis were generally quite precise after hydrolysis had been performed for 22 hours. If still more precise test results were required, then analysis samples that had been prepared under the same conditions, using the same sort of sealed test tubes, were subjected to hydrolysis for 22 hours, 48 hours, 72 hours, etc., and amino acid [values] that were obtained by interpolation were compared with the corrected values from their analyses at a hydrolysis time of zero.

(5) Preparation of the Analysis Sample: The test tube containing the completely hydrolyzed sample to be analyzed was removed [from the decomposing oven] and cooled. The test tube was then cut open at its narrow neck area and the hydrolyzed sample was dumped into a glass flask of the sort that is used to concentrate a specimen by evaporation. A vacuum evaporator was used to remove the hydrochloric acid. At each step of the operation, distilled water was added to dissolve any small amounts of amino acids that were present on the walls of the evaporating flask and wash them down to the flask's bottom. The operation to remove the hydrochloric acid was repeated several times (about three or four times) until all of it had been ultimately eliminated. The analysis sample was made up to its final volume by diluting it with a citrate buffer solution (pH 2.2) to prepare it for the next step, i.e., the analysis of its amino acid composition by the amino acid analyzer.

(6) Determination of the Amino Acid Content of the Analysis Sample: The amino acid content of the sample should be determined before it is analyzed, in order

to make sure that an excessively high content [of amino acids] in the sample will not cause the spectral absorption peaks to exceed the absorption range on the recording paper. The method that was utilized to ascertain the amino acid content of the sample in advance of its analysis was to compare the values that were obtained for the amino acid content of a 0.1 mL portion of a solution of the sample in question with the values that were obtained for 0.1 mL of a 1 mM standard amino acid solution (this was equivalent to a quantity of 1 μM when ninhydrin was employed as a color-developing [agent].) This would provide a standard for the quantity [of sample] to be used when the samples were injected into the amino acid analyzer.

(7) Analytical Results and Calculations: The areas occupied by the spectral absorption peaks that were produced on the recording paper as a result of the analysis were calculated using the triangulation method (i.e., the RH method). These areas were then compared to the analytical values that were found for the standard amino acids under the same conditions. By subjecting the analysis samples to the above procedures, the amino acid compositions and contents of the gelatins that were obtained from shark skin or skate skin could be determined.

G. Shark-Skin Gelatin and Skate-Skin Gelatin Bleached by Hydrogen Peroxide (H_2O_2)

Twenty grams of gelatin obtained from either shark skin or skate skin were placed in 100 mL of distilled water. The gelatin was completely dissolved [in] a 65° C water bath, then 2% or 6% (v/v) hydrogen peroxide was added to the gelatin solution for the purpose of bleaching it. By this means a gelatin solution that was clear and

transparent could be obtained. The resulting gelatin solution was then dried in air heated to 80° C (or, alternatively, in cold air), yielding shark-skin or skate-skin gelatin in the form of a snow-white powder. This bleaching step was carried out in order to investigate the feasibility of utilizing hydrogen peroxide to remove impurities from the gelatin so that it can be regarded as a food product suitable for consumption by the elderly and by children. [It needs to be stressed,] however, that no hydrogen peroxide can be allowed to remain in the gelatin, to be sure there are no [ill] effects on human health.

Results and Experiments

A. The Relationship Between the Extraction of Gelatin From Shark Skin or Skate Skin and Its Gel Strength

(1) The Relationship Between the Extraction of Gelatin From Shark Skin and the Gel Strength of the Gelatin

Figure 1 shows the results that were found with respect to [the gel strength of] the gelatin that was obtained when pre-treated shark skins were homogenized and then extracted at a temperature of 50°, 60°, 70°, 80°, 90°, 100°, 110°, or 120° C, etc. The vertical axis shows the gel strength of this shark-skin gelatin, while the horizontal axis shows the length of time the shark skin was soaked. From Fig. 1 it can be seen that 80° C is the ideal temperature for the extraction of gelatin from shark skin, as the pattern of variation that was observed for the gel strength of gelatin extracted from

shark skin at 80° C was linear with respect to the length of time the shark skin had been soaked; or, to put it another way, the gel strength of the gelatin increased with increasing soak time of the shark skin (from the first week to the seventh week). It is evident that gelatin derived from shark skin that was extracted at 80° C exhibited the greatest solubility, and as a result the gelatin extracted at that temperature /p. 40 showed the highest gel strength. Although the magnitude of the value for the gel strength of the gelatin cannot be taken to indicate in any absolute way the quantity of gelatin that can be extracted from the shark skin, it may be regarded as a bench mark for estimating the relative quantity of gelatin that can be extracted. Since the magnitude of its gel strength by no means represents the absolute quantity of gelatin that is extractable, comparisons had to be made between the gelatins that were obtained from the shark skins at each of the aforementioned extraction temperatures and the shark skins that were left behind as a residue of the extraction. The results of these comparisons will be discussed below. The pattern [of variation] was not so ideal, however, with respect to the gel strengths that were found for the gelatins extracted from shark skins at temperatures other than 80° C. Although the gel strength exhibited by shark-skin gelatin was observed to increase as the extraction temperature rose from 50° C to 80° C, the gel strength declined after that temperature level had been reached. Gelatin is a type of protein. Within its three-dimensional structure (*9, 11*) are [a] hydrophilic group and [a] hydrophobic group. When heat is applied in the process of extracting the gelatin, the increasing temperature gives rise

to denaturation (*9, 11*), as it causes the hydrophilic group to be broken down and the hydrophobic group to become exposed at the protein's surface. As a result, the solubility of the gelatin declines as the temperature of the heating rises, and this means that the amount of gelatin that is extracted will also fall off with an increase in the heating temperature.

(2) The Relationship Between the Extraction of Gelatin From Skate Skin and the Gel Strength of the Gelatin

Figure 5 presents the results that were found when skate skins were soaked for one to five weeks and then gelatin was obtained by homogenizing the pre-treated skate skins and extracting them at various temperatures ranging from 50° C to 90° C. The vertical axis of Fig. 5 shows the pattern of variation that was observed in the gel strength of the gelatins that were extracted from the skate skins at temperatures between 50° C and 90° C, while the horizontal axis shows the length of time (from one to five weeks) the skate skins were soaked. As can be seen from Fig. 5, maximum gel strength was exhibited by the gelatins that were extracted from skate skins at a temperature of 80° C after a soak time of up to three weeks. From this finding it is evident that 80° C is the optimal temperature for the extraction of gelatin from skate skin. In every case [except one], the gelatins that were extracted from skate skin at the other temperature levels — for example, 50°, 60°, and 70° C — showed their maximum gel strength when the soak time for the skate skin had been three weeks. The gelatin that was extracted from skate skin at 90° C, however, displayed its maximum gel

strength when the skate skin had been soaked for four weeks. Gelatin that was obtained from skate skin showed rising gel strength as the soak time for the skate skin increased up to three weeks, but with a longer soak time the gel strength underwent a decline. Now gelatin is a type of protein that may exhibit its greatest solubility at 80° C, and this is the reason it shows its maximum gel strength at that temperature. As the temperature at which the gelatin is extracted rises, the gelatin gradually undergoes denaturation, leading to a decline in its solubility and diminishing the amount of gelatin that is obtained.

B. The [Relationship Between] the Soak Time of Shark Skin and Skate Skin and the Ratio of Recovery of Gelatin From Them

(1) The Relationship Between the Soak Time of Shark Skin and the Ratio of Recovery of Gelatin From It

Figure 2 illustrates the relationship between the length of time shark skins were soaked and the ratio of recovery of gelatin from them. The vertical axis of this figure shows the ratio of recovery of gelatin from shark skin, and the horizontal axis shows the soak time of the shark skin. From Fig. 2 it can be seen that the ratio of recovery of gelatin from these shark skins also reached its optimal level when the extraction temperature was 80° C. The ratio of recovery of gelatin that was extracted from shark skin after a soak time of one week was approximately 79%, whereas the recovery ratio fell to only 59.5% when the gelatin was extracted from shark skin that had been soaked for two weeks. When the shark skin had been soaked for three weeks, /p. 42

however, the ratio of recovery for the gelatin rebounded back to around the 79% mark, and thereafter the recovery curve steadily rose [with increasing soak time]. The ratios of recovery that were cited above were percentages expressed on a dry weight basis. The results of these tests indicated that the ratio of recovery of gelatin from shark skin is approximately 17–25% of the wet weight of the shark skin. The results that were obtained for gelatin extracted from shark skin at a temperature other than 80° C were somewhat confusing. Slightly better results were found in another test that was conducted by the present worker with respect to the extraction of gelatin from the freshly peeled skins of skates that had just been offloaded from a ship and processed by the same method (see Fig. 6). A topic that may merit more investigation in the future is whether or not the pattern of variation that was noted for the extraction of gelatin from shark skin was related to the degree of freshness of the shark skin, its quality, or the position on the skin where the extraction was taking place.

(2) The Relationship Between the Soak Time of Skate Skin and the Ratio of Recovery of Gelatin From It

Figure 6 shows along its vertical axis the ratio of recovery of gelatin /p. 43 extracted from skate skin, while its horizontal axis shows the length of time (one to five weeks) the skate skin had been soaked. Optimal recovery of gelatin from the skate skin was observed when the extraction took place at 80° C, and the optimal soak time was found to be three weeks. With the exception of the gelatin that had been extracted at 60° C — a case in which the recovery curve exhibited a dissimilar pattern — the curves

for the recovery of gelatin all showed basically the same tendency regardless of the extraction temperature — i.e., whether it was carried out at 50°, 70°, 80°, 90° C, etc. Moreover, the ratio of recovery of gelatin from skate skin showed an increase as the length of the soak time rose from one to three weeks, whereas after the third week the recovery rate gradually dropped off. The ratio of recovery when the extraction was done at 30° C climbed from 59.5% for a soak time of one week to a level of 81% for a soak time of three weeks, but thereafter it declined. The ratios of recovery of gelatin from skate skin that are shown in Fig. 6 represent percentages that are expressed on the basis of the gelatin's dry weight. Now, assuming that the skin accounts for approximately 10% of the wet weight of a skate's body as a whole, and that the water content of the wet skin comes to approximately 75%, then about 25 g of skate skin, measured on a dry weight basis, can be obtained per kilogram of skate. If the ratio of recovery of gelatin from skate skin is calculated to be 80%, then 20 g of gelatin can be extracted per kilogram of skate. (Note: If we base the calculation on 1 kg of skate skin, then 200 g of gelatin can be obtained.) If we take one pound of gelatin to be worth about 280 New Taiwan Dollars — the price it currently sells for — then the value of the gelatin that can be extracted per kilogram of skate will be about 12 [New Taiwan] Dollars. At present skate sells for a very low price, so low that its skin is virtually regarded as a waste part. However, if a comparison is made between the gel that is obtained from skate skin and commercial gelatin ([marketed here under the name] Gelatin, U.S.P. Granular, by South America Minerals & Merchandise Corp.), it

will be found that a 2% [solution of] skate-skin gelatin has a gel strength of 26 g, whereas the gel strength of a 2% commercial gelatin [solution] is 14 g. From these figures it can be seen that gelatin produced from skate skin is of superior quality. To summarize the test results reported above, the extraction of gelatin from skate skin has excellent potential as a subsidiary fisheries processing industry, provided that sufficient quantities of the raw materials can be accumulated.

C. The Relationship Between the Extraction of Gelatin From Pork Skin and the Gel Strength of the Resulting Gelatin

Figure 3 shows how the gel strength of gelatin that was derived from pork skin varied [with soak time]. The maximum values for gel strength were obtained after one to three weeks [of curing the pork skins], but after the fourth week the gelatin exhibited a gradual decline in its gel strength. It can be seen that, when pork skin is soaked for a period of up to three weeks, the gelatin that is extracted exhibits its greatest gel strength. This pattern is quite similar to the pattern that was observed in connection with the extraction of gelatin from skate skin.

D. Results of Tests on Enzymatically Digested Shark-Skin Gelatin

(1) Shark-Skin Gelatin Digested With Papain

As can be seen in Fig. 4, the solid line with the black circles shows the results that were obtained [with respect to the gel strength] of a 20% [solution] of shark-skin gelatin that was digested with 0.01% papain. Once the shark-skin gelatin was being

acted upon by the papain, the gel strength of the gelatin gradually declined as the enzymic reaction time increased. By the time the gelatin solution had undergone four hours of enzymic reaction, its gel strength had already dropped to less than half the level that it had originally exhibited; i.e., it fell to about the 70 g level. The results that were obtained for gelatin that had been enzymatically digested for one hour showed that the gel strength had declined from 270 g to 212 g; this can be said to have represented only a minor reduction in the gelatin's gel strength. After this gelatin solution has been subjected to heat processing — i.e., after it has been heated at 100° C for 10 minutes, for the purpose of destroying the enzymic action — and then dried, the resulting product is ready to be utilized, possibly by being added directly to food products. In such cases it can serve as a binder that can be employed in ordinary food products or in refined or other types of fisheries food products.

(2) Shark-Skin Gelatin Digested With Pancreatin

As can be seen in Fig. 4, the solid line with the open circles shows the results that were obtained [with respect to the gel strength] of a 20% [solution] of shark-skin gelatin that was digested with 0.01% pancreatin. Once the shark-skin gelatin was being acted upon by the pancreatin, the gel strength of the gelatin steadily dropped off as the enzymic reaction time rose. By the time the gelatin solution had undergone four hours of enzymic reaction, its gel strength had already fallen to less than half the level that it had originally exhibited; i.e., it dropped to about 120 g. The results that were obtained for gelatin that had undergone enzymatic digestion for one hour revealed that

the gel strength had declined from 240 g to 196 g. After this gelatin solution has been subjected to heat processing — i.e., after the solution has been heated for 10 minutes at 100° C to destroy the enzymic action — and then dried, the resulting product is ready to be added directly to food products or to be otherwise utilized. In these instances it can serve as a binder that can be employed in ordinary food products or in refined or other types of fisheries food products. Gelatin solution that has been thus processed enzymatically has relatively little fishy smell or taste. It is possible that the fishy odor is reduced by lipase that is contained within the pancreatin, as this might be able to act on lipids remaining in the shark-skin gelatin. For this reason a gelatin solution that has been subjected to this enzymatic treatment is superior to one that has been treated with papain.

E. The Results of Amino Acid Analyses of Gelatins Derived From Shark Skin and From Skate Skin

(1) Amino Acid Analysis of Gelatin Derived From Shark Skin

The data listed in column A of Table 1 are the results that were obtained in an analysis of the amino acids present in gelatin derived from shark skin. When these results are compared with the amino acid composition that has been presented in the literature for gelatin derived from pork skin, it will be noted that the contents of several kinds of amino acids — for example, aspartic acid 9.8%, proline 11.2%, methionine 1.6%, phenylalanine 1.2%, lysine 6.5%, histidine 1.7%, and arginine 12% — differ [in shark-skin gelatin] from the contents that are seen for these amino acids in

pork-skin gelatin, but that other types of amino acids show very similar percentage contents [in the two types of gelatin]. The gelatin obtained from shark skin, however, does not contain hydroxyproline. Furthermore, the contents of the essential amino acids methionine (1.6%) and lysine (6.5%) are both higher in shark-skin gelatin than in pork-skin gelatin. The content of tryptophan was not analyzed at this time. In addition, column B of Table 1 lists the results that were obtained in an analysis of the amino acids present in a sample of shark-skin gelatin that had been bleached with hydrogen peroxide and then dried. When these results are compared with the amino acid composition that has been presented in the literature for gelatin derived from pork skin, it can be seen that the contents of several kinds of amino acids — for example, aspartic acid 13.1%, glutamic acid 13.4%, proline 10.1%, glycine 20.7%, phenylalanine 1.1%, lysine 5.2%, histidine 0.44%, and arginine 11.7% — differ [in this treated shark-skin gelatin] from the contents of these amino acids [in pork-skin gelatin]. Nevertheless, the contents of other types of amino acids are quite similar [in the two types of gelatin]. Hydroxyproline, moreover, is not present [in the gelatin derived from shark skin], but methionine has been destroyed on account of the oxidizing effect of the hydrogen peroxide. When this amino acid composition is compared with /p. 44 the amino acid composition that was observed for the shark-skin gelatin that had not been bleached, it can be seen that, with the exception of the slightly higher contents of aspartic acid (13.1%) and glutamic acid (13.4%), the contents of all of the other types of amino acids showed very close similarity.

(2) Amino Acid Analysis of Gelatin Derived From Skate Skin

The data listed in the column in Table 2 that is annotated with a 1 are the values for the amino acid composition of gelatin derived from pork skin. Pork-skin gelatin contains 19 kinds of amino acids. Tryptophan was not included among the amino acids that were analyzed. Hydroxyproline, however, is present in gelatin obtained from pork skin. The results shown in column A (annotated with a 2) in Table 2 are the [results of an] amino acid [analysis] of skate-skin gelatin. The contents of several types of amino acids — for example, aspartic acid 5.3%, serine 3.2%, glutamic acid 7.7%, proline 13.2%, glycine 22.1%, alanine 7.3%, isoleucine 1.0% leucine 1.9%, lysine 0.9%, and arginine 6.4% — differed from the contents seen in gelatin that is derived from pork skin. However, the sole [differences] that truly distinguish the amino acid composition of skate-skin gelatin are that its content of methionine (1.9%) is twice as high as the methionine content (0.9%) of pork-skin gelatin and its content of histidine (5.9%) is [about] eight times as high as the histidine content (0.78%) of pork-skin gelatin. Gelatin derived from skate skin does not contain hydroxyproline, and no analysis was made of tryptophan. As for cystine, this amino acid ought to appear in the form of cysteic acid in the chart produced by the analysis. Since no cysteic acid was revealed by the amino acid analysis, it can be concluded that gelatin derived from skate skin either may not contain cystine or contains it only at trace levels. The results shown in column B (annotated with a 3) in Table 2 are the [results of an] amino acid [analysis] of skate-skin gelatin that had been bleached with 2%

hydrogen peroxide. As can be seen from these data, the contents of some of the types of amino acids — for example, aspartic acid 6.8%, glutamic acid 10.3%, proline 12.0%, glycine 25.0%, alanine 8.0%, histidine 4.3%, and arginine 8.8% — were a little higher [in the bleached skate-skin gelatin], but the amounts of the other kinds of amino acids were quite similar [to their contents in unbleached skate-skin gelatin]. The contents of tyrosine and histidine both fell, to 0.12% and 4.3% respectively, and the methionine content also dropped, to 0.2%. Moreover, the amino acid composition of skate-skin gelatin that was bleached with 2% hydrogen peroxide was very similar to that found when 6% hydrogen peroxide was employed for bleaching. The results shown in column C (annotated with a 4) in Table 2 were obtained by an amino acid [analysis] of skate-skin gelatin that had been bleached with 6% hydrogen peroxide. Although the amino acid composition of the skate-skin gelatin subjected to this treatment was quite close to that of the skate-skin gelatin bleached with 2% hydrogen peroxide, two types of amino acids — i.e., methionine and tyrosine — were completely destroyed by the bleaching and did not show up in the analysis.

F. Results of Tests on Shark-Skin and Skate-Skin Gelatins Bleached With Hydrogen Peroxide (H_2O_2)

(1) Results of Tests on Shark-Skin Gelatin Bleached With Hydrogen Peroxide

Bleaching shark-skin gelatin with hydrogen peroxide at a concentration of either 2% or 6% has an advantageous effect on the gelatin as far as bleaching itself is concerned, but the results of the amino acid analyses (see column B in Table 1) make

it evident that the methionine contained by the gelatin is destroyed by this treatment. Therefore, when gelatin that has been derived from shark skin is being bleached, it is essential that relatively mild conditions be adopted for the treatment if destruction of the gelatin's amino acid contents is to be avoided. After shark-skin gelatin that had been bleached with 2% hydrogen peroxide had dried, a 3% measurement[9] of its restorability showed that the gel strength of the gelatin had dropped from 43 g to 18 g.

(2) Results of Tests on Skate-Skin Gelatin Bleached With Hydrogen Peroxide

Bleaching shark-skin gelatin with hydrogen peroxide at a concentration of either 2% or 6% also has an excellent effect on the gelatin as far as bleaching itself is concerned. However, as can be seen from the data in column B (annotated with a 3) of Table 2, the methionine content of skate-skin gelatin that has been bleached with 2% hydrogen peroxide was observed to fall from 1.9% to 0.2%, and both methionine and tyrosine were completely destroyed when the bleaching step employed 6% hydrogen peroxide. Furthermore, after skate-skin gelatin that had been bleached with 2% hydrogen peroxide had dried, a 3% measurement of its restorability showed that the gel strength of this gelatin, too, had dropped, from 45 g to 23 g. In view of the above, gelatin that is derived from skate skin should be bleached under fairly mild conditions to prevent the destruction of the nutrients it contains and to avoid a loss of gel strength.

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[9] Literally translated. The Chinese text does not specify what the 3% refers to, either here or below. Could the intended meaning be that the gel strength measurements were made on a 3% gelatin solution? (Translator's note.)

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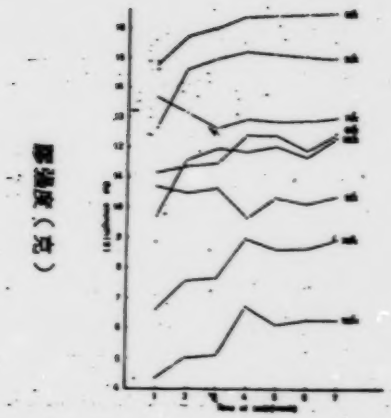
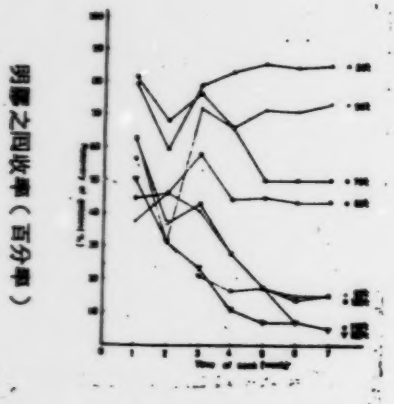
Figures and Tables



浸漬時間（週）

圖1 鯊魚皮之浸漬時間對於鯊魚皮明膠膠強度之影響

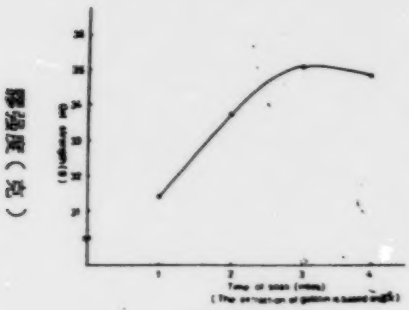
Fig. 1. The effect of soak time of shark-skin on the gel strength of shark-skin gelatin.



浸漬時間（週）

圖2 鯊魚皮之浸漬時間對於鯊魚皮明膠回收率之影響

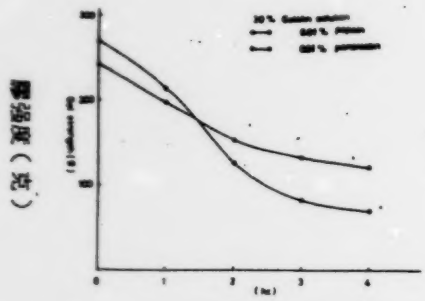
Fig. 2. The effect of soak time of shark-skin on the recovery of shark-skin gelatin.



浸漬時間（週）
（明膠以 80 ℃抽取）

圖3 豬皮之浸漬時間對於豬皮明膠膠強度之影響

Fig. 3. The effect of soak time of pork-skin on the gel strength of pork-skin gelatin (gelatin was extracted at 80°C)



反應時間（小時）

圖4 以 0.01 %之木瓜蛋白酶或胰蛋白酶處理鯊魚皮明膠。

1) 以 0.01 %木瓜蛋白酶於 *pH* 5.0，35.5 ℃與 20 %鯊魚皮明膠液反應。

2) 以 0.01 %胰蛋白酶於 *pH* 7.5，35.5 ℃與 20 %鯊魚皮明膠液反應，反應時間之間隔如圖所示。

Fig. 4. Treatments of shark-skin gelatin with 0.01% papain or with 0.01% pancreatin. 1)The reaction of 20% shark-skin gelatin solution with a 0.01% papain at pH 5.0, 35.5°C. 2) The reaction of 20% shark-skin gelatin solution with a 0.01% pancreatin at pH 7.5 35.5°C. The reaction time intervals were shown in figure.

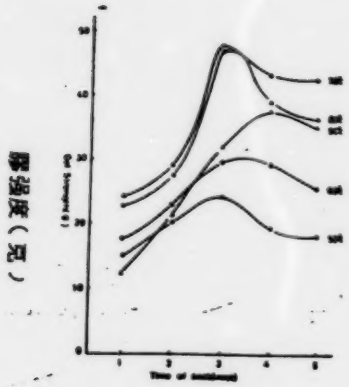


圖5 鮫魚皮之浸漬時間對於鮫魚皮明膠膠強度之影響

Fig. 5. The effect of soak time of skate-skin on the gel strength of skate-skin gelatin.

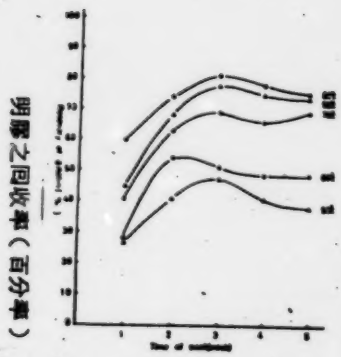


圖6 鮫魚皮之浸漬時間對於鮫魚皮明膠回收率之影響

Fig. 6. The effect of soak time of skate-skin on the recovery of skate-skin gelatin.

Amino Acid Composition			
Amino acid	Porkskin[1] gelatin (% by wt)	Sharkskin gelatin (% by wt)	
		A[2]	B[3]
Asp	6.7	9.8	13.1
Thr	2.2	3.0	3.2
Ser	4.2	3.7	3.6
Glu	11.4	11.5	13.4
Pro	16.4	11.2	10.1
Hypro	14.1	—	—
Gly	27.5	21.8	20.7
Ala	11.0	10.0	10.1
Cys	trace	—	—
Val	2.6	2.2	2.1
Met	0.9	1.6	0
Ile	} 5.1	1.8	1.6
Leu		2.6	2.2
Tyr	0.3	0.14	0.1
Phe	2.2	1.2	1.1
Trp	—	—	—
Lys	4.5	6.5	5.2
His	0.78	1.7	0.44
Arg	8.8	12	11.7

表1　鯊魚皮明膠之氨基酸成分（100%／100 g protein）

1) 豬皮明膠之氨基酸成分供比較之用
2) A：鯊魚皮明膠之氨基酸成分
3) B：以2%過氧化氫處理之鯊魚皮明膠之氨基酸成分

Table 1. Amino acid composition of shark-skin gelatin (100% / 100g protein). 1) Amino acid composition of pork-skin gelatin for comparison, 1) A:Amino acid composition of shark-skin gelatin. 3) B:Amino acid composition of 2% H_2O_2 treated shark-skin gelatin.

Amino Acid Composition				
Amino acid	Porkskin[1] gelatin (% by wt)	Skateskin gelatin (% by wt)		
		A[2]	B[3]	C[4]
Asp	6.7	5.3	6.8	7.9
Thr	2.2	2.6	3.4	3.3
Ser	4.2	3.2	3.9	4.0
Glu	11.4	7.7	10.3	10.8
Pro	16.4	13.2	12.0	12.0
Hypro	14.1	—	—	—
Gly	27.5	22.1	25.0	25.0
Ala	11.0	7.3	8.0	8.0
Cys	trace	—	—	—
Val	2.6	2.4	2.5	2.4
Met	0.9	1.9	0.2	—
Ile	} 5.1	1.0	0.94	0.9
Leu		1.9	1.8	1.7
Tyr	0.3	0.4	0.12	—
Phe	2.2	1.9	1.4	1.4
Trp	—	—	—	—
Lys	4.5	0.9	0.6	0.5
His	0.78	5.9	4.3	3.4
Arg	8.8	6.4	8.8	8.1

表2　鱏魚皮明膠之氨基酸成分（100%／100 g protein）

1)豬皮明膠之氨基酸成分供比較之用
2) A：鱏魚皮明膠之氨基酸成分
3) B：以2%過氧化氫處理之鱏魚皮明膠氨基酸成分
4) C：以6%過氧化氫處理之鱏魚皮明膠氨基酸成分

Table 2. Amino acid composition of skate-skin gelatin (100% / 100g protein). 1) Amino acid composition of pork-skin gelatin for comparison, 2) A:Amino acid composition of skate-skin gelatin, 3) B:Amino acid composition of 2% H_2O_2 treated skate-skin gelatin, 4) C: Amino acid composition of 6% H_2O_2 treated skate-skin gelatin.

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[10] No page number is shown for this reference. (Translator's note.)

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[11] The English spellings of Japanese names may vary widely, owing to a lack of standardization in the pronunciations of the *kanji* used in names. (Translator's note.)

English Abstract

Studies on the Extraction of Gelatin from Shark-skin and Skate-skin

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Gelatin is obtained by selective hydrolysis of collagen, the major intercellular protein constituent of white connective tissue of animal skins and bones. Gelatin consists of a mixture of water soluble proteins of high average molecular weight. It is capable of forming a firm gel in aqueous medium and, upon complete hydrolysis, yields various amino acids. During the course of this study, we found that shark-skin and skate-skin also contained a large quantity of collagen, it can be used as a raw material for the extraction of gelatin. An investigation was made to study the extraction of gelatin from shark-skin and skate-skin, moreover, the use of shark-skin gelatin treated with proteolytic enzyme as a food binder for food and fishery products. The optimum condition for the extraction of gelatin from shark-skin is desirable at *pH* 6.8 to 80℃ for 30 min., and the use of shark-skin soaked in saturated calcium hydroxide solution of three weeks. The optimum condition for the extraction of gelatin from skate-skin is desirable at 70°-80℃, *pH* 6.8, for 30 min., and the use of skate-skin soaked in saturated calcium hydroxide solution of three weeks. The gelatin solutions are then filtered to remove haze-forming bodies and dried in temperature-controlled ovens. The optimum condition for treatments of shark-skin gelatin with proteolytic enzyme are as follows : 1) 20% gelatin solution treated with a 0.01% papain solution at *pH* 5.0, 35.5℃, for 1 hr. 2) 20% gelatin solution treated with a 0.01% pancreatin solution at *pH* 7.5, 35.5℃, for 1 hr. After the incubation of 1 hr, heating the solution to 100℃ for 10 min. destroy enzyme activity, and then dried in oven, the product for food binder is good in quality. Amino acid of skate-skin gelatin was destroyed by bleaching with 2% H_2O_2 or 6% H_2O_2. The methionine content of 2% H_2O_2 treated skate-skin gelatin was reduced to 0.2% (untreated skate-skin gelatin contains 1.9%), and The tyrosine content of 2% H_2O_2 treated skate-skin gelatin was reduced to 0.12%. The methionine content and tyrosine content of 6% H_2O_2 treated skate-skin gelatin were destroyed completely by bleaching. It is desirable to use mild condition for bleaching of skate-skin gelatin and shark-skin gelatin.

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